

Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms *Actinomyces israelii*, *A. naeslundii*, *Candida albicans* and *Enterococcus faecalis*

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Abstract

Radcliffe CE, Potouridou L, Qureshi R, Hababbeh N, Qualtrough A, Worthington H, Drucker DB. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms *Actinomyces israelii*, *A. naeslundii*, *Candida albicans* and *Enterococcus faecalis*. *International Endodontic Journal*, 37, 438–446, 2004.

Aim To determine the resistance of microorganisms associated with refractory endodontic infections to sodium hypochlorite used as a root canal irrigant.

Methodology Two strains each of *Actinomyces naeslundii*, *Candida albicans* and *Enterococcus faecalis* were tested as late logarithmic phase inocula, against sodium hypochlorite adjusted to 0.5, 1.0, 2.5 and 5.25% w/v. Contact times used were 0, 10, 20, 30, 60 and 120 s. In the case of *E. faecalis*, additional experiments used contact times of 1.0, 2.0, 5.0, 10.0 and 30.0 min. Antimicrobial action was halted by sodium thiosulphate addition. Survivors were measured primarily using viable counts on drop plates. Additionally, pour plates were used to count low colony-forming units (cfu) and dilutions to 10⁻⁶ were used to count high cfu.

Results All concentrations of NaOCl lowered cfu below the limit of detection after 10 s in the case of *A. naeslundii* and *C. albicans*. However, *E. faecalis* proved to be more resistant to NaOCl. Using 0.5% NaOCl for 30 min reduced cfu to zero for both strains tested. This compares with 10 min for 1.0%, 5 min for 2.5% and 2 min for 5.25% ($P < 0.001$). Regression analysis for the dependent variable $\log_e(\text{count} + 1)$ with $\log_e(\text{time} + 1)$ and concentration as explanatory variables gave rise to a significant interaction between time and concentration ($P < 0.001$).

Conclusion The published association of *E. faecalis* with refractory endodontic infection may result, at least partially, from high resistance of this species to NaOCl. This does not appear to be the case with *A. naeslundii* or *C. albicans*.

Keywords: *Actinomyces israelii*, *Actinomyces naeslundii*, antimicrobial, *Candida albicans*, endodontics, *Enterococcus faecalis*, sodium hypochlorite.

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Introduction

Numerous studies have analysed the microflora of infected dental root canals (Gomes *et al.* 1994, 1996, Khemaleelakul *et al.* 2002, Peters *et al.* 2002). Nevertheless, there is limited information with respect to which microorganisms persist and survive after completion of root canal treatment (Hancock *et al.* 2001). However, certain groups of microorganisms seem to be associated with persistent endodontic infections.

Actinomyces

Sjögren & Sundqvist (1987) investigated the role of infection on the prognosis of endodontic therapy by following-up teeth that had undergone cleaning and filling during a single appointment. They found that *Actinomyces* species were present in three cases with failed endodontic treatment whilst no other specific bacteria were implicated. Baumgartner & Falkler (1991) found that *Actinomyces* species were amongst the most prominent bacteria cultured from the 10 root canals examined. Additionally, Sundqvist (1994) reported that *Actinomyces* species were isolated from approximately 15% of cases. Although *Actinomyces* isolates are recovered from teeth with necrotic pulps, they are mainly associated with cases of failed root canal treatment with asymptomatic periapical lesions (Byström et al. 1987).

Candida albicans

Although yeasts have occasionally been reported in untreated cases (Sen et al. 1995), they have been more associated with cases of failed endodontic treatment (Nair et al. 1990). Molander et al. (1998) also looked for *Candida albicans* and detected three isolates from a total of 100 canals with chronic apical periodontitis and two from 20 root canal samples without signs of apical periodontitis. To what extent *Candida* species may be of significance in the persistence of periapical infection is unknown. However, it may be significant that such species are present and may not respond to conventional cleaning and shaping procedures in the same way as other organisms.

Enterococcus

Although these bacteria are usually found in low numbers in untreated infected root canals with necrotic pulps (Sundqvist 1992), they may thrive and multiply if ecological parameters change. *Enterococcus faecalis* has been isolated from 38% of teeth that had recoverable microorganisms (Sundqvist et al. 1998). Furthermore, Molander et al. (1998) examined the microbiological status of root filled teeth with apical periodontitis and found that facultative anaerobes predominated with enterococci being the most frequently isolated group. Hancock et al. (2001) examined root filled teeth with persistent periapical radiolucencies, and found that as well as *Enterococcus*, other genera, viz. *Peptostreptococcus*, *Actinomyces*, and *Streptococcus*, predominated. Additionally, *Enterococcus* has been isolated from 47% of root canals in which treatment had failed (Pinheiro et al. 2003).

Generally, facultative anaerobes are less susceptible to anti-microbial measures than are anaerobes and are therefore more likely to survive root canal treatment unless cleaning and shaping procedures are of the highest standard. The treatment resistance of enterococci in the root canal has long been recognized (Moller 1966). Significantly, calcium hydroxide has been shown to be ineffective in killing *E. faecalis* in root canals (Haapasalo & Orstavik 1987, Reit & Dahlen 1988) and this is predictable because this microorganism is able to grow at high pH (Drucker & Melville 1971).

Sodium hypochlorite is also effective in aiding the mechanical flushing of debris from root canals. McComb & Smith (1975) showed that irrigation with 6% sodium hypochlorite resulted in canals that were almost completely free of debris. This compound dissolves organic matter (Spånberg 2002) and has a broad spectrum of antimicrobial activity. Thus, sodium hypochlorite effectively eliminates microbes from root canals (Byström & Sundqvist 1983, 1985, Jeansonne & White 1994) and also kills bacteria within open dentinal tubules (Buck et al. 2001). Although sodium hypochlorite exhibits cytotoxicity, it is controversial whether or not it reduces the ability of the surviving tissue to recover.

The reasons why *Enterococcus faecalis* and other microorganisms should be associated with persistent infection are ill understood. However, *E. faecalis* is known to be a resistant species. It can withstand certain chemical agents, including some antibiotics, which would be highly toxic to other organisms. Its persistence in endodontic infection might be aided by an enhanced resistance to sodium hypochlorite (Gomes et al. 2001).

Thus, the aim of this study was to evaluate, *in vitro*, the effectiveness of 0.5, 1.0, 2.5 and 5.25% sodium hypochlorite on the viability of some endodontic organisms associated with refractory endodontic infections, over a range of time intervals.

Materials and methods

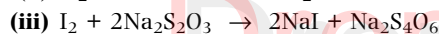
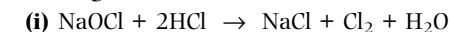
Culture of microorganisms

Actinomyces naeslundii NCTC 27038 and NCTC 12104 were cultured on Fastidious Anaerobe agar (FAA; Lab

M, Bury, UK) with added sterile horse blood (Oxoid, Basingstoke, UK) from stored -80°C cultures. The *A. naeslundii* strains were both cultured in a Compact M anaerobe workstation (Don Whitley, UK). *Candida albicans* NCYC 1467 and R1 were cultured on Sabouraud Dextrose agar (SDA; Lab M) from stored broth cultures. *Enterococcus faecalis* NCTC 775 and E10e were cultured on Colombia blood agar (CBA; Lab M) from cultures stored at -80°C . *Candida albicans* NCYC 1467, *Actinomyces naeslundii* ATCC 12104 (=NCTC 10301) and ATCC 27038, and *E. faecalis* ATCC 19433 (=NCTC 775) were kindly donated by J. Verran (Manchester Metropolitan University). *Candida albicans* R1 and *E. faecalis* E10e were isolated from patients at the University Dental Hospital of Manchester.

Standardization of sodium hypochlorite

Sodium hypochlorite was purchased from Sigma (Poole, UK) as a solution, which was 'no less than 8.38% w/v'. The chemical is unstable even stored at $+4^{\circ}\text{C}$ where it slowly decomposes to yield NaCl and molecular oxygen. Therefore, the strength of the solution had to be determined by titration. The following reactions were used:



Specifically, NaOCl was adjusted to a nominal $0.00349 \text{ mol L}^{-1}$ and the test reagents were prepared as 0.01 mol L^{-1} solutions. Consequently, 1.0 mL NaOCl (ca. $0.00349 \text{ mol L}^{-1}$) was added to 1.0 mL 0.01 mol L^{-1} KI, followed by 1.0 mL 0.01 mol L^{-1} HCl. The free iodine liberated and seen as a yellow colour was then titrated with 0.01 mol L^{-1} $\text{Na}_2\text{S}_2\text{O}_3$. Analyses were repeated five times and results averaged.

Preparation of chemicals

Sodium hypochlorite solution was diluted to give accurate concentrations of 0.5, 1.0, 2.5 and 5.25% to be tested against the microbes. NaOCl was neutralized after testing using sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) (Sigma). Two concentrations were required, 1.93% for the neutralization of 0.5% NaOCl and 3.86% for the neutralization of concentrations greater than 1.0% NaOCl. Phosphate buffered saline (PBS) (Sigma) of pH 7.1 was used for serial dilutions and in control experiments at a concentration of 0.01 mol L^{-1} , prepared as per the manufacturer's instructions.

Growth curves

Before experiments were carried out growth curves were determined for each strain to be studied. Thus, knowledge of the expected numbers at certain times and knowledge of when the microbes were in log phase of growth was acquired.

Test microorganisms were inoculated from plate culture into 50 mL of an appropriate broth (Fastidious Anaerobe broth, Lab M, for *A. naeslundii* and *E. faecalis*; Sabouraud Liquid Medium, Lab M, for *C. albicans*). Cultures were incubated overnight in a shaking water bath at 37°C . Prior to sealing cultures of *A. naeslundii*, they were exposed to an anaerobic atmosphere which provided CO_2 . Following overnight culture, 0.2 mL of inoculum was transferred to 19.8 mL of the appropriate broth. The bottle was mixed briefly on a spinmix (GallenKamp, Loughborough, UK). Readings of absorbance at 550 nm were taken using a spectrophotometer at time 0 h and every hour after up to 10 h. In between performing absorbency measurements, cultures were incubated in the shaking water bath. After removal of *A. naeslundii* culture samples, each hour, the bottle was re-sealed inside the anaerobic workstation to maintain an anaerobic atmosphere. After each spectrophotometric reading had been taken, the sample was serially diluted. To determine colony-forming units (cfu) present, $20 \mu\text{L}$ aliquots were inoculated onto appropriate agar for incubation and viable counts determination.

Growth curves showed that the late log phase for each organism was attained between 3 and 4 h after the initial inoculation, consequently cultures were left to grow for this amount of time before experiments proceeded to ensure the microbes were actively growing when they were stressed with NaOCl.

Preparation of starter cultures for experiments

Test microorganisms were grown overnight as above to provide a liquid culture. After overnight growth, 0.2 mL liquid culture was added to 19.8 mL fresh broth (appropriate to the microorganism) in a sterile 50 mL conical flask. Test cultures were then grown for 3.5 h in the shaking water bath at 37°C to allow cells to achieve log phase.

Exposure to test chemical

Aliquots (0.2 mL) of starter culture were added to 9.8 mL NaOCl (0.5, 1.0, 2.5 or 5.25%), bottles were

mixed using a spinmix to ensure immediate exposure of organisms to the chemical. Aliquots (1 mL) were removed after 10, 20, 30, 60 and 120 s and immediately transferred to 9 mL $\text{Na}_2\text{S}_2\text{O}_3$ for neutralization of NaOCl. Aliquots (0.2 mL) from each neutralized solution were transferred to agar plates and spread using a sterile glass spreader. Plates were incubated at 37 °C (anaerobically for *A. naeslundii*) for 2 days before colonies from surviving cells were counted. The whole procedure was repeated four times for each strain at each concentration of NaOCl.

Controls

In order to assist interpretation of results from the main experiment, controls were utilized:

1. A test microorganism positive control which tested for microbial growth without chemical stress, i.e. NaOCl was replaced with sterile PBS and the rest of the experiment was carried out as above.
2. A neutralizing agent toxicity control which confirmed that $\text{Na}_2\text{S}_2\text{O}_3$ was not toxic under the 'in-use' conditions, i.e. NaOCl was replaced with $\text{Na}_2\text{S}_2\text{O}_3$ and the rest of the experiment continued as above.
3. A sodium thiosulphate effectiveness control: sodium hypochlorite (0, 2.5 and 5.25% w/v) was mixed with sodium thiosulphate (1.93 and 3.86% w/v respectively) then inoculated with *E. faecalis* inoculum. Viable counts were performed on samples taken at times 0, 0.5, 1, 2, 5, 10 and 30 min, for each strain, per NaOCl concentration.
4. A negative control which tested the sterility of reagents and medium being used, i.e. microbial culture was replaced with sterile broth and the rest of the experiment was carried out as above.

Viability

Plate cultures were examined for growth under a plate microscope 24 and 48 h after exposure to the test chemical. The number of viable colonies was recorded and the cfu per mL calculated. The viable counts were the mean of four repeat experiments performed for each strain–time–concentration permutation tested.

Additional experiments

1. Due to the very low numbers of survivors found (*vide infra*) with strains of *Actinomyces naeslundii* and *Candida albicans*, additional experiments were performed for these species. Rather than using just

0.2 mL of the exposed culture, the whole test mixture (microbe plus chemical plus neutralizing agent) was transferred to five aliquots of 20 mL molten agar, without added blood (2.0 mL test mixture per 20 mL agar). The molten agar was immediately poured into sterile Petri dishes, allowed to set and then incubated as above. Colonies throughout the agar for the whole volume of test mixture could thus be counted.

2. Due to the relative resistance of *E. faecalis* to treatment with NaOCl (*vide infra*), the experiment was repeated using serial dilutions of the test mixture in order to obtain countable numbers of colonies after incubation. Rather than inoculating 0.2 mL from the test mixture directly onto a plate it was first diluted (from 10^{-1} to 10^{-6}). From each dilution 20 µL was inoculated onto an agar plate and then incubated as above.

Statistical analysis

The data collected were entered onto a spreadsheet and statistically analysed using the software packages Stata and SPSS/PC+ v10.0. For each strain a regression model was fitted to the dependent variable, which was viable count, for the independent variables of time and concentration, including an interaction term. In order to take the clustering of samples into account, the regression analysis was conducted using 'Stata'. The latter package provides robust estimates of the standard error of the time coefficient.

The variables, time and viable count, were transformed by taking natural logs in base e (and adding unity to avoid taking logs of zero) so that this transformation produced a linear relationship between $\log_e(\text{time} + 1)$ and $\log_e(\text{count} + 1)$, per concentration. The rest of the analysis was conducted using SPSS/PC+ v10.0 where descriptive data and plots of the data were produced.

Results

Control experiments

The data from control experiments for all test microorganisms were identical:

1. The test microorganism positive controls showed that all strains grew if PBS replaced NaOCl so that any lack of growth could be attributed to NaOCl.
2. The neutralizing agent positive controls revealed that $\text{Na}_2\text{S}_2\text{O}_3$ was not toxic to any test microorganism at the 'in-use' concentration.
3. The sodium thiosulphate effectiveness control showed that the survival rate for *E. faecalis* was at least

95% except in two experiments when it was 94 and 92% respectively. Data presented in Table 1 prove that sodium thiosulphate can very swiftly neutralize sodium hypochlorite so that un-neutralized sodium hypochlorite does not remain active beyond stated contact times.

4. The growth negative controls produced no detectable growth when inoculum was absent which confirmed that the various media and reagents used were sterile, in other words not contaminated.

Actinomyces naeslundii

Both strains behaved identically. All concentrations tested of NaOCl, including 0.5% w/v, proved effective against these strains within as little as 10 s.

Candida albicans

This oral yeast proved very susceptible to the action of sodium hypochlorite. All strains tested gave the same results. Each concentration of sodium hypochlorite proved effective against every strain at all contact times. Over 9 million cfu were decreased to below the limit of detection by as little as 0.5% sodium hypochlorite within 10 s contact time.

Enterococcus faecalis

This species proved significantly more resistant to sodium hypochlorite than the other species tested. Both strains behaved similarly (Table 2). As the concentration of sodium hypochlorite was increased, the time taken to reduce numbers of cfu below the limit of detection decreased. After 30-min contact time, even 0.5% NaOCl had reduced viable counts below the limit of detection. When higher concentrations were employed, less time was required although even 5.25% NaOCl was not completely effective after 1.0 min although it was by 2.0 min. The complex relationships between viable counts, contact time and concentration of NaOCl are shown graphically in Figs 1 and 2. These show data for *E. faecalis* E10e and NCTC 775 respectively and the data for different strains are extremely similar. Data for both strains were pooled in order to obtain Fig. 3 which depicts the relationship between concentration of sodium hypochlorite and time necessary to attain zero viable counts. Clearly there was a strong curvilinear relationship between concentration and time taken to attain zero viable counts.

Contact time (min)	<i>Enterococcus faecalis</i> E10e		<i>Enterococcus faecalis</i> NCTC 775	
	2.5% NaOCl + 1.93%Na ₂ S ₂ O ₃	5.25% NaOCl + 3.86%Na ₂ S ₂ O ₃	2.5% NaOCl + 1.93%Na ₂ S ₂ O ₃	5.25% NaOCl + 3.86%Na ₂ S ₂ O ₃
0.0	100 ^a	100	100 ^b	100
0.5	99	99	99	99
1.0	99	98	99	99
2.0	99	98	98	98
5.0	98	97	97	96
10.0	98	97	97	96
30.0	96	96	95	96

See text for experimental details.

^a100% corresponds to 4.70×10^9 cfu mL⁻¹.

^b100% corresponds to 4.65×10^9 cfu mL⁻¹.

Table 1 Effectiveness of sodium thiosulphate in neutralizing sodium hypochlorite expressed as percentage survivors

Contact time (min)	<i>Enterococcus faecalis</i> E10e NaOCl				<i>Enterococcus faecalis</i> NCTC 775 NaOCl			
	0.5%	1.0%	2.5%	5.25%	0.5%	1.0%	2.5%	5.25%
0.0	100 ^a	100	100	100	100 ^b	100	100	100
0.5	83	53	35	21	85	72	60	43
1.0	62	35	21	5	74	50	40	9
2.0	50	25	3	0	61	28	14	0
5.0	33	10	0	0	47	17	0	0
10.0	13	0	0	0	23	0	0	0
30.0	0	0	0	0	0	0	0	0

Survival values are calculated from mean cfu⁻¹ data. See text for experimental details.

^a100% corresponds to 4.90×10^9 cfu mL⁻¹.

^b100% corresponds to 4.60×10^9 cfu mL⁻¹.

Table 2 Effectiveness of sodium hypochlorite in reducing viability of *Enterococcus faecalis* expressed as percentage survivors

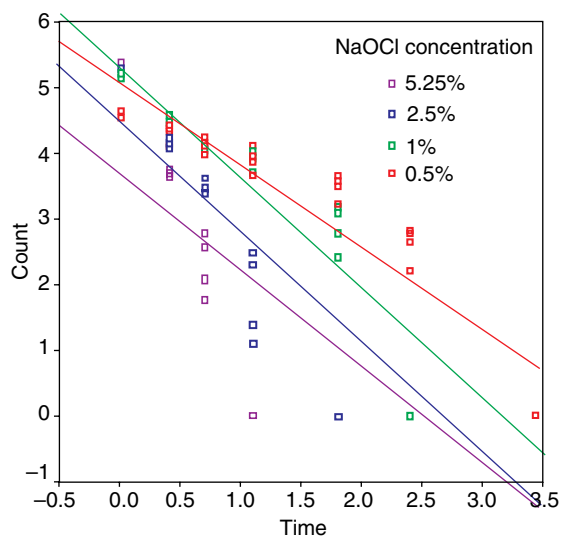


Figure 1 The relationship between $\log_e(\text{time} + 1)$ for viability of *Enterococcus faecalis* E10e at various concentrations of NaOCl.

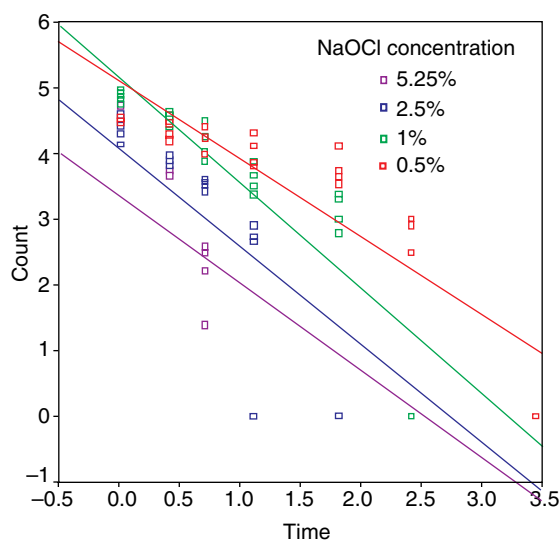


Figure 2 The relationship between $\log_e(\text{time} + 1)$ for viability of *Enterococcus faecalis* NCTC 775 at various concentrations of NaOCl.

Statistical analysis

In the case of *A. naeslundii* and *C. albicans*, data were so clear as not to require statistical tests.

For *E. faecalis* E10e (Table 2) using a concentration of 0.5% NaOCl, an exposure time of 30 min reduced viable count to zero, compared with 10 min for 1.0%, 5 min for 2.5% and 2 min for 5.25% ($P < 0.001$). Data are shown in Fig. 1 for the relationship between

$\log_e(\text{time} + 1)$ and $\log_e(\text{count} + 1)$ per concentration of NaOCl. It can be seen that there is a significant difference between the mean number of viable counts recovered ($P < 0.001$).

Regression analysis for the dependent variable $\log_e(\text{time} + 1)$ with $\log_e(\text{count} + 1)$ and concentration as explanatory variable gave rise to a significant interaction between time and concentration ($P < 0.001$). It is clear from Table 2 and Fig. 1 that there is no significant difference between the mean of viable counts recovered for all concentrations at time intervals of 0.0 min (start) and 0.5 min. However, the differences between the concentrations become apparent at longer time intervals (1.0, 2.0, 5.0, 10.0, 30.0 min).

Results for *E. faecalis* NCTC 775 were similar to those of *E. faecalis* E10e, with a strong relationship between the mean of viable count and time, for each concentration of NaOCl (Table 2 and Fig. 2). Significant differences between the concentrations were also apparent for the longer time intervals ($P < 0.001$), with the highest concentration (5.25%) reducing the viable counts to zero in 2.0 min compared with 30.0 min for the lowest concentration (0.5% NaOCl). Therefore, a strong curvilinear relationship exists between concentration and time taken to attain zero viable counts (Fig. 3).

Discussion

Choice of strains

Although species of both *Actinomyces* (Byström et al. 1987) and *Candida* (Nair et al. 1990) have been

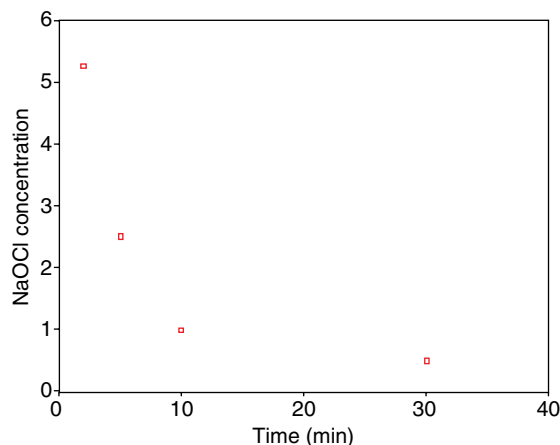


Figure 3 Relationship between NaOCl concentration and time necessary to attain zero viable counts for *Enterococcus faecalis* E10e and NCTC 775.

implicated in endodontic flare-ups, *E. faecalis* has been most often associated with infection in treated canals (Pinheiro *et al.* 2003). The latter species has been studied extensively with respect to the efficacy of endodontic irrigants (Heling & Chandler 1998, Ayhan *et al.* 1999, Gomes *et al.* 2001). One of the strains used in this study, viz. *E. faecalis* NCTC 775, has previously been used in a study on the antibacterial effects of endodontic irrigants (Vahdaty *et al.* 1993).

Strength of sodium hypochlorite

The stock solution was found to be 10% less concentrated than when tested upon initial receipt. Thus NaOCl should ideally be assayed before dilution if accurate concentrations are required. Nevertheless, there is no consensus regarding the 'correct' concentration for use in endodontic procedures (Cheung & Stock 1993) because concentrations used have ranged from 0.5 to 10.0% w/v (Matsumoto *et al.* 1987). In practice the concentration of NaOCl chosen is a compromise between its antibacterial activity and its cytotoxicity. Concern over cytotoxicity led Byström & Sundqvist (1983) to recommend use of 0.5% NaOCl in order to minimize cytotoxicity yet retain antimicrobial efficacy. Recently, doubt has been cast on the effectiveness of 0.5% NaOCl (Siqueira *et al.* 1998), which is certainly reinforced by the present findings when resistant microorganisms are involved.

Use of sodium thiosulphate

Few if any studies have shown whether this chemical can rapidly and effectively neutralize NaOCl. This is important if contact times are to be believed because failure to neutralize NaOCl would effectively prolong contact times with NaOCl. It was also essential to confirm that $\text{Na}_2\text{S}_2\text{O}_3$ is not itself toxic at 'in-use' concentrations which would also have negated conclusions drawn from test data.

Action of NaOCl

The effectiveness of NaOCl against *A. naeslundii* even with 0.5% w/v concentration in contact for only 10 s is not entirely unexpected. This is because the high E_h , let alone high pH, creates very hostile conditions for a species which is normally grown under anaerobic conditions. Nevertheless, Georgopoulou *et al.* (1994) found that two *A. naeslundii* strains resisted 2.5% NaOCl for 5 and 15 min respectively. One explanation

for the difference from the present findings may be the differing methodologies used. The sensitivity of *C. albicans* to NaOCl is less expected because this yeast can survive quite hostile conditions, including survival for up to 12 min in pure water. However, related yeasts are destroyed industrially using NaOCl, which rapidly kills cells. In the case of *E. faecalis*, a recent study by Gomes *et al.* (2001) gives similar results to the present study regarding the time taken to kill cultures of this species. They have shown that 5.25, 2.5, 1.0 and 0.5% NaOCl kills *E. faecalis* ATCC 29212 after contact times of <0.5, 10.0, 20.0 and 30.0 min respectively. Small differences in findings can probably be explained in terms of differing methodological detail. In the present study, a more dilute inoculum was used, different media were employed (Columbia Agar in place of Brain Heart Infusion) and different strains of *E. faecalis* (NCTC 775 and E10e in place of ATCC 29212). The direct exposure method used in this study appears to be a simple straightforward and practical laboratory test. Nevertheless, the clinical efficacy of NaOCl should also be viewed in the light of complex root canal anatomy (Orstavik & Haapasalo 1990), the polymicrobial nature of root canal infections and the presence of biofilms (Spratt *et al.* 2001). Biofilms elsewhere are known to affect and limit solute diffusion. Another factor to be considered is the presence of the smear layer, which may hinder irrigant that is attempting to enter dentinal tubules (Sundqvist *et al.* 1998). Additionally, interaction of NaOCl with tissue fluids, blood, dentine and other organic debris can reduce its effectiveness. Chemo-mechanical preparation is a short-term procedure, and NaOCl remains in the root canal for only a few minutes but may penetrate into the tubules. Thus, the antibacterial effectiveness of NaOCl within the root canal might be expected to be a function of concentration and contact time as found in the present work (Siqueira *et al.* 2000). In other words, an irrigating solution that is effective against a single microorganism *in vitro* may not be so effective *in vivo* in the dental root canal (O'Hara *et al.* 1993).

Finally, it should be remembered that the antimicrobial action of NaOCl is only one reason why it is used endodontically. Root canal irrigants must display additional properties, which include high detergent power, low surface tension, ease of handling and high proteolytic and tissue solution abilities (D'Arcangelo *et al.* 1999). In the case of NaOCl this irrigant does not leave noxious residues as it is unstable and breaks down to form oxygen and sodium chloride, which are naturally found in the body.

Conclusion

The relatively high resistance to NaOCl of *E. faecalis* may result in its surviving dental root canal treatment and subsequently being associated with refractory infection. However, this does not account for the suggested association of *Actinomyces naeslundii* or *Candida albicans* with such infections.

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